EXPERIMENTAL HISTOPLASMOSIS IN MONKEYS

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EXPERIMENTAL HISTOPLASMOSIS IN MONKEYS

by
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In 1955, Courtois, Segretain, Mariat and Levaditi described a spontaneous mycosis in the Cynocephalic monkey of Guinea. It was a question of a large-sized histoplasmosis, that is to say, one belonging to that group of histoplasmosis in which the intracellular saccharo-mycetes have a diameter of 8 to 12μ.

Since we had recently had occasion to isolate two strains of large sized Histoplasma capsulatum from human lesions, it appeared to us to be interesting to:

a. Attempt to produce this experimental disease in the Cynocephalic monkey by using these human strains.

b. Ascertain, in particular, if the chlamydospore obtained in vitro could reproduce in this animal an affection identical to the one that the Cynocephalic monkey can present spontaneously.

Procedure. An adult Cynocephalic monkey, under observation for five months in the monkey section of the Pasteur Institute and kept isolated in an individual cage.

On 16 May, it was inoculated subcutaneously at the root of its tail with a Histoplasma capsulatum culture.

This strain was isolated from a cutaneous human histoplasmosis observed on a patient of Dr. Ba in Kaolack.

Two downy colonies that showed, under direct examination, numerous characteristic chlamydospores of Histoplasma capsulatum, were removed, cut up, crushed, and then suspended in Hank's solution.

The evolution of the affection in the monkey was torpid. In the third month a fistulization was observed at the place of inoculation, and, in the fourth month -- on 15 September -- the presence of an ulceration was noticed in the vicinity of the right paw.

We should have liked to prolong our observation as far as possible, but on 14 November, during a bioptic puncture of the liver, the monkey suffered a fatal syncope when subjected unfortunately to a hypertension.

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RESULTS

I. Autopsy.

A monkey of the male sex, visibly emaciated and displaying:

1. At the junction of the one third interior and of the one third middle of the tail, an edematous infiltration that increased the size of this tail over a section 2 to 3 cm. long. The skin in the vicinity of this area presented a livid appearance dotted with several pin-head erosions or ulcerations.

2. In the vicinity of the right second toe, an ulceration about 5 mm. in diameter, sanious and vegetative at its base, with regular edges, not turned but rimmed with an edematous infiltration.

3. Small punctiform ulcerations, resembling blood specimen puncture scars, spread out over the left upper member and connected by lymphangitic tracks.

An inguinal adenopathy, predominating on the right, with ganglions the size of a large bean, without periadenitis.

A longitudinal incision in the tail lesion showed, under an infiltrated skin, a necrotic tissue, pultaceous in appearance, punctated with hemorrhagic areas. The lesion was strictly limited between the skin and the aponeurosis, without reaching the subaponeurotic and underlying osteomuscular layers.

Direct examination of a section from the lesion, stained with methylene blue, showed a mass of large-sized histoplasmas, usually free, rarely enclosed in histiocytes. The section was rich in associated germs. Implantations were made in a potato-carrot culture-medium. Some pulverizations were inoculated intraperitoneally in white mice, a guinea-pig, three kantchoulis, and subcutaneously, at the root of the ear, in a rabbit.

The complete autopsy, moreover, revealed:

1. A mesenteric adenopathy with several congestive ganglions.

2. A spleen slightly increased in size, but presenting, in a diffuse manner, whitish granulations, non-raised, the size of a pin-head, visible over the entire external surface.

When sectioned, a soft spleen with hyperpasia of the white pulp; the granulations are less visible on the inside of the splenic parenchyma.

3. A large, congestive liver, but primarily steatosic without any lesion in its center.

4. Superficial hemorrhagic dots at the base of the lung, probably correspondings to agonal lesions.

5. All the other organs, except the nervous system, when examined in detail revealed no macroscopic lesion. Most of the organs were removed.

II. Histopathological examination.

Removal of the tail (skin plus subcutaneous cellular tissue).

The epidermis presented an appreciably normal appearance: there was an orthokeratosic hyperkeratosis, a mucous body of average thickness,

epidermic papillae without appreciable lesion, and a normally pigmented base.

The dermis was rather sclero-inflammatory, but contained the addition, in particular, of pilosebaceous follicles. The dermic sclerosis was a diffuse, not very cellular, collagenous sclerosis. Some perivascular, perineural and perisudoriparous infiltrates, composed of lympho-histiocyte elements were found in the superficial dermis, but this inflammatory development appears to be connected with the large hypodermic lesions.

In fact, characteristic lesions of large-sized histoplasmosis were present in the hypodermis and separated from the superficial dermis by a thick collagen strip. In fact, a granuloma, whose dominant characteristic is the intensity of the plasmodial reaction, was found there. It is a question of large elements, varying in size from 50 to 200μ in diameter, rounded or polygonal with acidophilic cytoplasm, containing thirty or more hyperchromatic nuclei, arranged very frequently around the periphery of the element, rarely grouped in the center. The plasmodia contained a varying amount of oval bodies, rarely rounded, of the average size of a lymphocyte. They were chromophobic, but contained a granulous hematoxyphilic substance in a mass in the center of the saccharomycetes or, most frequently crescent-shaped.

The Hotchkiss-Mac Manus stain dyes these elements intensely around the periphery and irregularly in the central formations.

Sections frozen and stained with Sudan III, with Black Sudan B, showed the existence of lipoidic substances that most frequently filled the center area.

The other inflammatory elements of the granuloma were lymphocytes, histiocytes, plasmocytes, macrophages, some of which enclose the saccharomycetes individually, and, primarily, neutrophilic polynuclears that form, in certain places, genuine small abscesses and a few rare polyeosinocytes.

Finally, the granuloma, rich in collagen fibers, was very abundantly vascularized with angiomatous aspects in places. The thrombosis of numerous capillary vessels must be noted.

Removal of the paw lesions:

Here there was an ulceration of the skin in the vicinity of which the same granuloma is found, but with an obviously purulent appearance, especially in the vicinity of the superficial areas. The vascular thromboses were more numerous, the giant cells less numerous and less bulky. There was a clear preponderance of the histiocytic reaction.

The other organs:

Liver: Presence of a large amount of palustral pigment with reticular hyperplasia.

Spleen: Massive hyperpasia of the white pulp, simulating the macroscopic granulous lesions. Presence of palustral pigment, likewise.

The other organs do not show any histologic lesion.

III. Subcultures.

On the basis of yeast-like elements removed from the vicinity of

the cutaneous lesions of the monkey, an attempt was made to isolate the parasite in vitro.

The cultures were perfectly successful in a potato-carrot medium, and, on the fifteenth day, the typical chlamydospores of Histoplasma capsulatum were identified in the white down that was removed and examined on a slide under a cover glass under a phase-difference microscope.

In conclusion:

Histoplasma capsulatum, in its large-sized variety, under its chlamydospore aspect, was inoculated in the Cynocephalic monkey. This parasite causes a typical cutaneous mycosis, during the development of which the histoplasma recovered its yeast-like appearance. Subcultures were perfectly successful.

The monkey, therefore, is sensitive to the large-sized, African variety of Histoplasma capsulatum. The anatomico-pathological picture is superposable on the one obtained from human lesions, and its passage into the animal does not at all modify the cultural characteristics of the parasite.

SUMMARY

Experimental histopasmosis in monkeys.

The authors succeeded in infecting Cynocephalus monkeys with two strains of large form H. capsulatum isolated from human lesions, and subsequent subcultures. In Cynocephalus monkeys the anatomico-pathological picture is the same as in human lesions; the animal passage does not modify the cultural properties of the parasite.

BIBLIOGRAPHY

Baylet, R., Hubert and Quenum, C., "Index histopasmique en A.O.F.," Bull. Soc. Path. exot., Vol. 51, 1958, No. 4, pages 582-589.

Courtois, G., Segretain, F., Mariat, F. and Levaditi, J.C., "Mycose cutanee a corps levuriformes observee chez des singes africains en captivite," Ann. Inst. Pasteur, Vol. 89, 1955, page 124.

Quenum, C., L'histoplasmose africaine a grandes formes, University of Paris thesis, 1958.

FIGURE CAPTIONS:

Fig. 1. Ulcerated inflammatory granuloma, very rich in histoplasmas.

Fig. 2. The tail, incised longitudinally in the vicinity of the point of inolculation, shows an edematous, necrotic and hemorrhagic infiltration. The lesion is very rich in large-sized histoplasma saccharomycetes, in direct examination.

Fig. 3. The granuloma with giant cells containing histoplasma saccharomycetes in the light patches. (Mag. 220X).

Fig. 1. — Granulome inflammatoire ulcéré très riche en histoplasmes.

Fig. 2. — La queue incisée longitudinalement au niveau du point d'inoculation montre une infiltration œdémateuse, nécrotique et hémorragique. La lésion est très riche en levures d'histoplasme à grandes formes à l'examen direct.

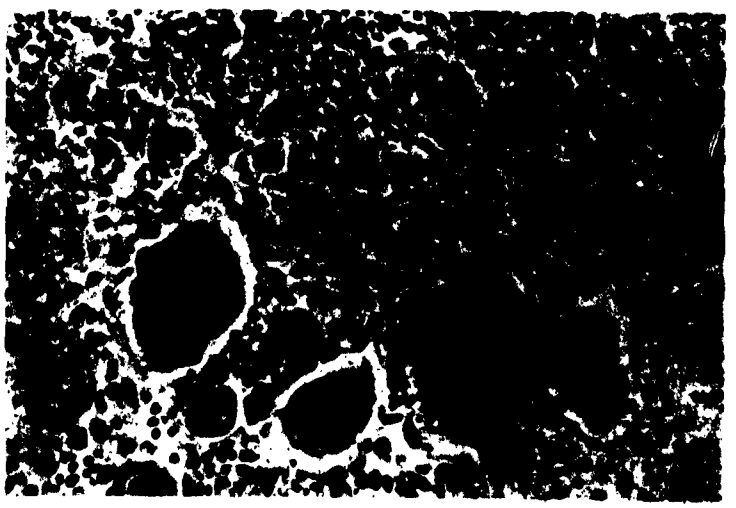

Fig. 3. — Le granulome avec cellules géantes contenant en clair les levures d'histoplasme (Gr. 220).